

Resistance factors and pathological process

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INDIVIDUAL REACTIVITY OF GRANULOCYTIC SYSTEM OF NEWBORN CALVES AND ITS ROLE IN PATHOGENESIS OF INFLAMMATORY DISEASES OF RESPIRATORY AND GASTROINTESTINAL TRACTS

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Abstract

Inflammatory diseases of the respiratory and gastrointestinal tracts are the actual problem of veterinary medicine. Innate or natural immunity plays the leading role in the initial reactions to microbial agents contaminating sterile mucous membranes of newborn animals. It is implemented through the activation of cellular and humoral factors of nonspecific resistance. Leukocyte concentration, leukogram, content of cationic proteins in neutrophils, blood serum lysozyme activity (BSLA) were studied in 20 red-motley calves in 1 hour after their birth on the days 2, 5-7 and 14-15 of life, together with an impact of these indices on the terms of appearance and clinical course of inflammatory diseases of the respiratory and gastrointestinal tracts, under conditions of a large dairy complex environment (Voronezhpishcheprodukt Co Ltd, Novousmansk district, Voronezh region) in 2014. Blood sampling was implemented in the morning before feeding from the jugular vein. Body temperature, pulse and respiratory rate, state of the visible mucous membranes, time of appearance and intensity of sucking reflex, the presence and nature of cough, dyspnea, nasal expiration, reaction to palpation of the larynx, trachea, intercostal spaces, the abdominal wall, percussion and auscultation of the chest were determined in calves. The markers of intestinal inflammation are soluble protein, erythrocytes (hemoglobin), leukocytes (leukocyte elastase), pH. They were identified in feces by dry-chemistry method during the same period and also selectively at the age of 1-1.5 months. According to clinical and hematological indices the animals were retrospectively divided into 2 groups: group A with leukocyte concentration of $(11.0-18.3) \times 10^9/l$ and segmentonuclear neutrophils (SN) more than $4 \times 10^9/L$ at birth; group B with leukocyte concentration of $6.5-11.3 \times 10^9/l$ and SN lesser than $3.9 \times 10^9/l$ at birth. Granulocyte blood concentration dynamics in calves of these groups significantly differed both among themselves and from physiological dynamics (adaptive norm) during the first 15 days of their life. This was determined by various phases of adaptation syndrome (mobilization, resistance, exhaustion) and various possibilities of calves' granulocytic system reaction to irritants impact according to J. Wilder's law of initial values (1957). The increased blood level of SN and rod neutrophils (RN) in animals of group A at birth indicates existing activation of bone marrow and may not significantly increase in response to a rather intensive stimulus. Initial level of SN and RN in calves of group B was close to the physiological one, it significantly increased on the day 2 and decreased by the days 5-7 of life. Cationic protein concentration and BSLA in all the calves during the research period were lower than the indices typical of the animals of this age. Diarrhea developed in all the calves on the day 2 of life. Its duration in animals of groups A and B was 8.6 ± 1.1 and 4.2 ± 0.6 days, respectively. When diarrhea symptoms disappeared, intestinal inflammation markers were identified in calves' feces during 1-1.5 months. During the first 14 days of life the first bronchitis symptoms (induced cough) were registered in all the animals. The course of the disease was light in most of the calves. The mechanisms of changes of granulocytic system reactivity, leading to its decompensation and respiratory tract inflammation development, are discussed.

Keywords: calves, leukocytes, segmentonuclear neutrophils, cationic proteins, feces analysis, phases of biological systems reactivity, inflammatory diseases.

Inflammatory diseases of the respiratory and gastrointestinal tracts are still an urgent problem of veterinary medicine: they affect from 50 to 100 % of calves, starting from the first days of life [1, 2]. Numerous studies of newborn's

immune status mainly cover the issues related to formation of the colostral and lymphocytic components of the adaptive immune system and its disorders in case of inflammatory diseases [3-5]. At the same time, innate or natural immunity plays a leading role in the initial reaction of the organism to the microbial agents contaminating the sterile mucous membranes of newborn animals [6-8]. It is implemented through the activation of the cellular and humoral factors of nonspecific resistance: segmentonuclear neutrophils (SN's), macrophages, as well as the antimicrobial and regulatory substances which are secreted by them and, in turn, initiate a cascade of reactions involving lymphocytes (immune response as such) [9, 10].

SN's represent the first barrier of the antimicrobial protection which is provided by high content of such cells in blood in the newborn in the first hours of life and in first-yield colostrum [11-14]. SN activation is accompanied by the intensification of metabolic processes (respiratory explosion) and the secretion of large amount of antibacterial substances differing in chemical nature [15-17]. Such stereotyped reaction occurs to metabolites of microbial origin, for example, lipopolysaccharides (LPS's) of gram-negative bacteria [18], as well as to hormones, blood and tissue cell mediators, and various kinds of radiation [19-22].

On mucosal surface and in mucosal secretions, SN's perform their function not only by phagocytosis, but also due to the release of DNA and bactericide granules forming neutrophil extracellular traps [23, 24]. SN malfunction on the mucosal surface (often due to the loss of bactericide potential during migration from bone marrow, in the circulatory channel) is considered as one of the factors associated with the formation of pathological microbial communities leading to the development of dysbioses and inflammation of various localization [25, 26].

We were the first who identified the patterns of quantitative changes for SN's and band neutrophils (BN's) in peripheral blood in single-breed cattle within 2 weeks after birth and substantiated the relationship between the natural immunity cellular component reactivity phase at birth and the pattern of pathology development.

The work was aimed at investigating the individual dynamics of the content of granulocytic-series blood cells and their bactericide activity in calves from day 1 to day 15 of life in connection with the onset and subsequent course of respiratory and gastrointestinal tract inflammatory diseases.

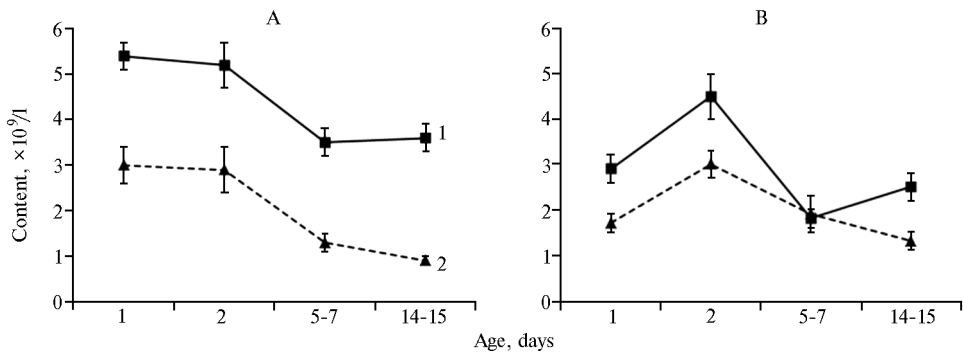
Technique. The studies were carried out in Voronezhpishcheprodukt LLC (Novousmanskyy District, Voronezh Region) in March—May 2014. A total of 20 red-and-white calves were randomly selected. The animals were kept in a preventorium by 5-6 heads per cage within 10-20 days. Then they were transferred to the group cages of a calf shed (by 5-8 heads) where they stayed until the age of 2-4 months. Newborn calves received colostrum from their mothers from suckling bottles 3 times per day. Within 10 days, colostrum (then milk) was given in the amount of $\frac{1}{10}$ of animal weight; from day 10 to day 20, this amount was equal to $\frac{1}{5}$, and from day 21, whole milk substitute or skim milk was used. The calves were trained to eat hay from the age of 10-12 days and concentrated feedstuffs from the age of 18-20 days.

The animals were examined 1 hour after birth (before the first colostrum feeding), on days 2, 5-7 and 14-15 of life. Body temperature, pulse and respiratory rate were measured; condition of visible mucous membranes, time of appearance and intensity of sucking reflex, and the presence and nature of cough, dyspnea and nasal discharges were assessed; palpation of the larynx, trachea, intercostal spaces and abdominal wall was carried out along with auscultation and

percussion of the chest. Blood was sampled in the morning, before feeding, from the jugular vein using a vacuum system with ethylene diamine tetraacetic acid (EDTA); feces samples were taken directly from the rectum. The total number of leukocytes in blood was measured using a Micros-60 analyzer (Horiba ABX, France); leukogram was calculated using a standard method after Romanowsky blood smear staining. Cationic protein concentration in neutrophils was assessed by the microscopic study of the blood smears stained using Fast Green FCF and by counting of a total cytochemical factor (TCF) [27]. BSLA was determined as described [28]. The rapid analysis of feces was conducted by a «dry-chemistry» method [29] with the use of 10EA test strips for urine analysis (Arkray, Japan) to test the following: presence of soluble protein, erythrocytes, leukocytes (leukocyte elastase) bilirubin (semiquantitative estimation), as well as pH. The clinical study and analysis of feces of 1-1.5 month old animals were also carried out on a selective basis.

Statistical processing of the results was carried out using a correlation analysis and *t*-test for independent variables in the Statistica v. 8.0 software (StatSoft Inc., USA).

Results. The amount of leukocytes and neutrophils in blood in calves on day 1 of life varied from $6.5 \times 10^9/l$ to $18.3 \times 10^9/l$ (total leukocytes), from $1.6 \times 10^9/l$ to $7.2 \times 10^9/l$ (segmentonuclear neutrophils) and from $1.0 \times 10^9/l$ to $4.4 \times 10^9/l$ (band neutrophils).



The dynamics of segmentonuclear (1) and band (2) neutrophil content in peripheral blood in red-and-white calves from day 1 to day 15 of life: A — group A, B — group B (Voronezhpishcheprodukt LLC, Novousmanskyy District, Voronezh Region, 2014).

With regard to the amount of leukocytes in blood at birth, the animals were divided into two groups: group A with values from $11.0 \times 10^9/l$ to $18.3 \times 10^9/l$ and SN more than $4.0 \times 10^9/l$ ($n = 11$) and group B with values from $6.5 \times 10^9/l$ to $11.3 \times 10^9/l$ and SN less than $3.9 \times 10^9/l$ ($n = 9$). The dynamics of segmentonuclear and band neutrophil content in blood in calves from both groups demonstrated a close correlation (correlation coefficient $r = +0.76$ at $p < 0.05$, see Fig.).

SN amounts in calves from groups A and B statistically significantly differed not only on day 1 of life, but also at later observation stages (Table).

Cationic protein concentration in neutrophils (according to TCF) in calves on day 1 was also characterized by significant variability (from 0.22 to 0.72) and was substantially lower than in adult animals (1.02-1.37) [30]. At later dates, both increase and decrease of TCF were noted in different animals as compared to the value on day 1, however this parameter did not correlate with the amount of segmentonuclear or band neutrophils and did not reach the age norm.

BSLA concentration throughout the whole period of study was within

0.1-0.3 rg/ml, while the norm for calves of such an age is 0.3-0.5 rg/ml [32, 33]. This indicates a low bactericide potential of SN's in the examined animals [34].

The content of segmentonuclear (SN) and band (BN) neutrophils ($\times 10^9/l$) in blood in red-and-white calves from groups A and B, differing in the amount of leukocytes at birth, within the period from day 1 to day 15 of life ($X \pm x$, Voronezhpishcheprodukt LLC, Novousmanskyy District, Voronezh Region, 2014)

Age, days	SN		BN	
	Group A (n = 11)	Group B (n = 9)	Group A (n = 11)	Group B (n = 9)
1	5.4 \pm 0.33*	2.9 \pm 0.32	3.0 \pm 0.39*	1.7 \pm 0.17
2	5.2 \pm 0.48	4.5 \pm 0.45 ^d	2.9 \pm 0.54	3.0 \pm 0.34 ^d
5-7	3.5 \pm 0.27*	1.8 \pm 0.24 ^d	1.3 \pm 0.20 ^d	1.9 \pm 0.39 ^d
14-15	3.6 \pm 0.34*	2.5 \pm 0.33 ^d	0.9 \pm 0.12	1.3 \pm 0.22

* p < 0.05 as compared to a similar indicator in Group B.

^d p < 0.05 as compared to the previous period of study within the group.

The clinical condition of calves from groups A and B was significantly different. Particularly, diarrhea duration was 8.6 \pm 1.1 and 4.2 \pm 0.6 days, respectively; time of appearance of the first signs of bronchitis (induced cough) was 3.7 \pm 0.7 and 7.6 \pm 1.6 days of life, respectively. In all calves from group B, the course of bronchitis was mild, while 2 of 11 calves in group A demonstrated a moderate course. Upon the onset of diarrhea symptoms, erythrocytes (++++), leukocytes (++) , soluble protein (++, +++) and, in several cases, bilirubin at pH 5-7 were found in feces. These parameters were not normalized with the disappearance of clinical signs of diarrhea. All intestinal inflammation markers (soluble protein, erythrocytes, leukocytes) persisted, however, manifestations were less pronounced (+, ++). This was observed in calves not only at the age up to 15 days, but also 1-1.5 months, which, in our opinion, indicates the transformation of acute intestinal inflammation into chronic one.

In the first days of calf's life, all organs and systems actively adapt to extrauterine existence. Within days 5-7, physiological changes are observed in various parameters of vital activity and metabolism [33, 35]. The granulocytic system of calves in the first 24 hours is characterized by the high number of leukocytes (up to 11 $\times 10^9/l$) and segmentonuclear neutrophils (up to 64 %), which rapidly decreases starting from day 2 of life [11]. The percentage of band neutrophils from day 1 to day 15 varies from 4 to 5 % without significant peaks and troughs. The absolute values of these parameters depend on the breed of animals and season of study [35, 36].

The analysis of our results in groups A and B has revealed two different patterns of granulocyte dynamics other than physiological one. Group A demonstrated initially high SN and BN content lasting within 2 days with subsequent decrease by days 5-7 and stabilization by day 15. The initial number of these cells in group B was 1.8 and 1.7 times lower, but it sharply increased on day 2, which reflected the release of the cells from bone marrow in response to its irritation. Because a diarrhea syndrome developed in all calves on day 2, we think that it is legitimately to associate the response of the granulocytic system with an inflammatory process in the intestine. However, it is still questionable why it is so different in the calves of the same breed, sex and age, which are kept in the same conditions.

The individual features of the organism response are determined by the initial values of parameters under study [37]. The dependence of a response to a stimulus on a value of the studied parameter in initial state was first described in 1957 by J. Wilder as a "Law of Initial Value" (LIV) [37]. According to the LIV, a change in any parameter (difference between final and initial values) is the less, the higher the initial value is. The higher the initial level is, the more often paradoxical responses to stimuli, which usually increase the studied parameter, are

observed, i.e. an increase in the initial parameter leads to a reduction in capabilities for the further stimulation of the function.

With regard to the granulocytic system, this is expressed in the fact that initially high number of SN's in blood (irrespective of the causes) cannot significantly increase even in response to rather intensive stimuli. Because peripheral blood leukocytes represent a transient pool reflecting the intensity of cell migration from bone marrow into tissues, a change in their number characterizes the reserve of mature cells and proliferative activity of the bone marrow. The high content of BN's in blood and appearance of earlier precursors indicate the significant activation of bone marrow, however, its proliferative activity, with retention of humoral stimulation, will expectedly pass, in accordance with a general adaptation syndrome phase sequence, from the mobilization stage to the resistance stage and then to the exhaustion (areactivity) stage, which will manifest as a decrease in leukocyte content in peripheral blood with the remaining or new focus of inflammation. It is the variant which was observed in the group A calves demonstrating a decrease in granulocyte number by days 5-7 against the background of diarrhea and appearance of the first symptoms of bronchitis.

In spite of the polyethiological nature of diarrhea in calves [1, 3], it is always combined with an intestinal inflammation and wall permeability disorder [38], and with an increase in translocation of intestinal microflora endotoxins, in particular, LPS, into the system blood flow [39]. At the same time, load on the functions of the liver and SN's binding LPS increases. According to the results of feces analysis, the cessation of diarrhea in calves was not accompanied by the elimination of enteritis. The inflammatory process in the intestine remained in the subclinical form which was verified only by laboratory testing. We believe that chronic endotoxemia is the main cause maintaining the continuous additional load both on the SN function and the whole granulocytic system [40-44]. Against such background, any, even nonspecific, stimulus, for example, stress in case of regrouping of calves or abnormal parameters of microclimate, may cause the immune system to pass from the resistance stage to the decompensation stage [37, 42]. This will manifest as inflammatory diseases affecting, first of all, the respiratory passages and lungs which are always in contact with pathogenic and opportunistic pathogenic environmental microflora [45]. In spite of the fact that the mucous membranes of the respiratory passages contain the biggest part of the SN marginal pool [7], their bactericide activity may not be sufficient for efficient protection due to a decrease in such activity as far back as in peripheral blood, which is indicated by the results of cationic protein and BSLA studies.

In calves from group B, granulocyte content on day 1 was close to a physiological norm, therefore, in response to humoral stimulation, a pronounced increase in SN's and BN's in accordance with the LIV was noted on day 2. It is likely that the reactive potential of the system was one of the reasons behind the fact that the duration of diarrhea in these calves was half as long as in animals from group A, and the first signs of bronchitis appeared much later.

Thus, at birth, the innate immunity cellular component reactivity in clinically healthy calves is in different phases. The causes of it are undoubtedly associated with a functional and metabolic status of mother cows during the pregnancy period, however, the molecular mechanisms of humoral influence on the granulocytic system of the fetus are almost unknown. As in case of animals with depressed reactivity, the calves having a highly reactive granulocytic system at birth are susceptible to respiratory and gastrointestinal tract inflammatory diseases, the only differences are in the onset time and severity of the pathological process. Therefore, the strength of the combined specific (bacterial) and nonspe-

cific (stress) factors activating the granulocytic system of newborn calves turns out to be excessive and is rapidly transferred at cellular and tissular levels from the stage of mobilization and resistance to the stage of exhaustion, i.e. immunodeficiency. Because the granulocytic component is functionally linked to the lymphocytic, macrophage and humoral components, immunodeficiency may manifest in different forms and degrees. The use of immunostimulators for correction of secondary immunodeficiencies, as well as vaccines with adjuvanticity, often leads to exacerbations of inflammatory diseases or even death of animals because the stimulation of the system at the exhaustion stage causes a failure of adaptation. In our opinion, pathogenetically substantiated methods for prevention and therapy in such cases can be those aimed at releasing the immune system from excessive (by frequency and strength) irritation by specific and nonspecific agents.

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